

# A Radula Muscle Preparation from the Gastropod, *Kelletia kelletii*, for Biochemical Assays

BY

HOWARD M. FEDER

Hartnell College, Salinas, California 93901

AND

REUBEN LASKER

United States Bureau of Commercial Fisheries

California Current Resources Laboratory, La Jolla, California 92037

(1 Text figure)

THE MUSCLE BUNDLES, the odontophore muscle complex or radula muscle, which move the molluscan radula have been physiologically examined in isolated preparations from two species of carnivorous gastropods: *Busycon canaliculatum* (LINNAEUS, 1758) (HILL, 1956, 1958), a snail from the east coast of North America, and *Buccinum undatum* LINNAEUS, 1758 (FÄNGE & MATTISON, 1957, 1958), a snail common to European waters. These authors found that the muscle contracts under the influence of acetylcholine and is relaxed by 5-hydroxytryptamine and that rhythmic contractions of the muscle occur when acetylcholine and 5-hydroxytryptamine are added together. FÄNGE (1963) and FEDER & ARVIDSSON (1967) showed that aqueous extracts of the sea stars *Henricia sanguinolenta* (MÜLLER, 1776), *Marthasterias glacialis* (LINNAEUS, 1758) and *Asterias rubens* LINNAEUS, 1758 also induce strong contractions in the isolated radula muscle of *Buccinum undatum*. Because of the usefulness of this kind of radula muscle preparation for bioassay we sought one from a gastropod of the American west coast. This paper reports on the preparation and use of the radula muscle from the carnivorous snail *Kelletia kelletii* (FORBES, 1850) for biochemical assays.

*Kelletia kelletii* were collected by means of SCUBA at a depth of about 20 m on sandy ocean bottom, near the Scripps Institution of Oceanography, La Jolla, California. The bathymetric range of *K. kelletii* is known to extend from 18 to 62 m (ABBOTT, 1954). Some of the *Kelletia* were subsequently shipped to Hartnell College and used in experiments there. The radula muscle was prepared and

mounted by a modification of the techniques of FÄNGE & MATTISON (1958), FÄNGE (1963), MATTISON & ARVIDSSON (1966), and FEDER & ARVIDSSON (1967) for isolating the radula muscle complex of *Buccinum undatum*. The final *Buccinum* muscle preparation included part of the proximal proboscis sheath which served for suspension of the muscle. *Kelletia* radula muscles prepared in this manner tended to behave erratically and showed a decreased sensitivity as compared to *Buccinum* radula muscles. This fact necessitated that the *Kelletia* radula muscle and the inner epithelial lining of the proboscis sheath be teased free of the sheath; the resulting preparation was more responsive and less erratic. The preparation was then suspended directly from the epithelial lining to the recording lever (Figure 1). The chamber was a plastic 30 cc syringe with the outlet enlarged to permit a rapid outflow when it was flushed. A lever with an arm 18 cm long and counterweighted with 2.0 g was used. The writing stylus was a fine glass rod of capillary size with a rounded tip. The muscle was mounted to the lever at its proximal end by a cotton thread through the epithelial tissue and to the glass rod at its oral end by a small loop from the radula sheath tissue. During perfusion sea water was either removed by aspiration or allowed to overflow the chamber. All other details of the experimental setup were the same as those described for radula muscle preparations of *Buccinum undatum* by FEDER & ARVIDSSON (1967).

Specimens of the sea star *Pisaster ochraceus* (BRANDT, 1835) were taken either from pilings off the pier at Scripps Institution of Oceanography, or from rocky reefs off La

Jolla, and from intertidal areas in Monterey Bay, California. The sea star *Pycnopodia helianthoides* (BRANDT, 1835) was collected in intertidal areas in Monterey Bay. Extracts were obtained by the same procedures used by FEDER & ARVIDSSON (1967) for the preparation of *Marthasterias glacialis* test solutions. Living sea stars were washed in tap water, frozen, and then thawed; the resultant cloudy exudate was collected and centrifuged. The clear supernatant fluid was used in all tests.

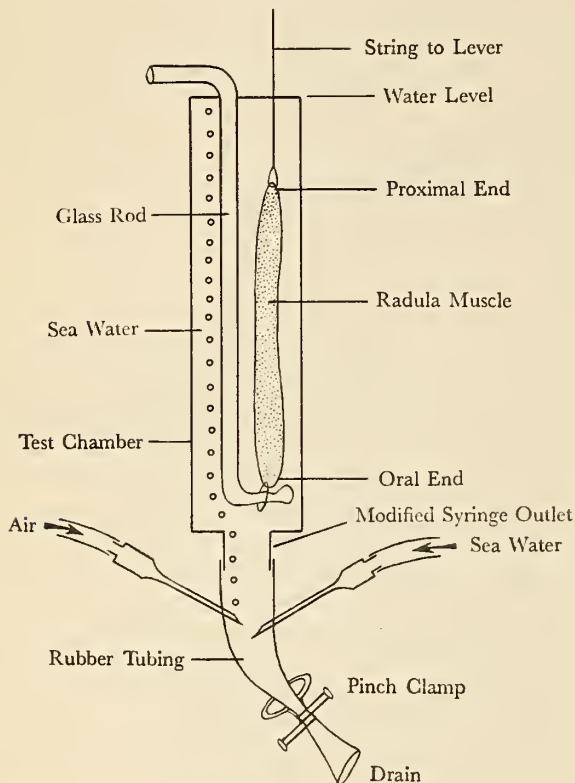


Figure 1

Diagram of the mounted radula muscle preparation

The *Kelletia* radula muscle contracted in the presence of acetylcholine of which the final concentration was  $5 \times 10^{-8}$  g/ml. Extracts of *Pisaster ochraceus* also caused the radula muscle of *Kelletia* to contract; the strength of the contractions varied roughly with amount of extract added. After addition of acetylcholine or *P. ochraceus* extract the contraction could be reversed immediately by

$3 \times 10^{-7}$  g/ml (final concentration) of 5-hydroxytryptamine. Strong, rhythmical contractions followed the initial relaxation. Similar responses of *Kelletia* muscle to *Pycnopodia* extracts were found in preliminary experiments. Responses, however, were always of greater magnitude to *Pycnopodia* than to *Pisaster ochraceus*, whether as contractions in response to the extracts or rhythmic contractions in response to the extracts after addition of 5-hydroxytryptamine. The amplitude of muscle contraction after addition of *Pycnopodia* extract was 2 to 3 times greater than that caused by addition of identical volumes of *Pisaster ochraceus* extract.

Sensitivity of muscles varied with time; in general, older preparations, 24–48 hours old maintained in a water bath at 10° C, were more sensitive. In addition, muscle preparations responded only when a certain "threshold" concentration (40 microliters in a final volume of 30 ml of sea water) of *Pisaster* extract was reached. An addition of sea star extract 5 times the threshold amount (i. e. 200 microliters in a final volume of 30 ml of sea water) frequently caused an extremely strong contracture from which the muscle often did not recover when the sea water in the bath chamber was renewed. Thus, like *Buccinum* muscle preparations, responses were quantitative only within a narrow range of extract additions.

The successful isolation of the radula muscle complex of *Kelletia kelletii* and its subsequent use in assay increases the number of available preparations of this type to three; *Busycon canaliculatum* and *Buccinum undatum* represent the other two species. The *Kelletia* preparation is very similar to that of *Buccinum*, and the responses of the radula muscles of the two species also are similar. It is probable that other carnivorous snails have radula muscles suitable for physiological and pharmacological studies.

## ACKNOWLEDGMENTS

This work was supported by a National Science Foundation Research Participation Grant to one of us (H. M. F.), and was accomplished at the Bureau of Commercial Fisheries, Fishery-Oceanography Center, La Jolla, California. We thank Mr. Daniel Tam of Hartnell College and Mr. William Vlymen of the Scripps Institution of Oceanography for their very able technical assistance, and Mr. George Crozier of the Scripps Institution of Oceanography and Mr. Robert Bowers of the Scripps Tuna Oceanography Research Program for collection of experimental animals.

## LITERATURE CITED

ABBOTT, ROBERT TUCKER

1954. American seashells. Princeton, New Jersey. D. van Nostrand Co., Inc.; xiv + 541 pp.; 100 figs.; 40 plts.

FÄNGE, RAGNAR

1963. Toxic factors in starfishes. *Sarsia* 10: 19 - 21

FÄNGE, RAGNAR &amp; ARTUR MATTISSON

1957. Rhythmical activity of a smooth muscle rich in haematin compounds. Swedish conference on cell research. *Ark. f. Zool.* 11: 112 - 113

1958. Studies on the physiology of the radula muscle of *Buccinum undatum*. *Acta Zoologica* 39: 53 - 64

FEDER, HOWARD M. &amp; J. ARVIDSSON

1967. Studies on a sea star (*Marthasterias glacialis*) extract causing avoidance reactions and radula muscle contractions in a gastropod (*Buccinum undatum*). *Ark. Zool.* 19: 369 - 379

HILL, ROBERT BENJAMIN

1956. Regulation of rhythmic activity in two types of red muscle in *Busycon canaliculatum*. *Anat. Rec.* 125: 119
1958. The effects of certain neurohumors and of other drugs on the ventricle and radula protractor of *Busycon canaliculatum* and on the ventricle of *Strombus gigas*. *Biol. Bull.* 115: 471 - 482

MATTISSON, ARTUR &amp; J. ARVIDSSON

1967. Some effects of electrical stimulation and exogenous metabolites on the contractile activity and the ultrastructure of the radula-muscle of *Buccinum undatum*. *Zeitschr. für Zellforsch.* 73: 37 - 55

